Activities of Lipoprotein Lipase and Hepatic Lipase on Long- and Medium-Chain Triglyceride Emulsions Used in Parenteral Nutrition

Olivier Lutz, Thierry Lave, Anny Frey, Zahia Meraihi, and André C. Bach

Prolonged parenteral nutrition frequently includes lipid emulsions. This report investigates how emulsions containing triacylglycerols of different molecular weight affect the rate of clearance in vivo and the activity in vitro of the two enzymes responsible for this clearance: diaphragm lipoprotein lipase (LPL) and hepatic endothelial lipase (HL). Whatever their molecular weight, the triacylglycerols of the emulsions were hydrolyzed by LPL and HL. However, the reaction was faster with medium-chain triglycerides (MCT) than with long-chain triglycerides (LCT). To be active, LPL required the presence of serum (apolipoprotein CII); for maximum activity less serum was required for MCT then for LCT. In the case of HL, serum inhibited the effect on LCT but not on MCT. However, hydrolysis of emulsified triacylglycerols by LPL and HL required the presence of albumin as a transporter of the fatty acids released. Less albumin was needed for maximum activity with MCT than with LCT. In vivo, although MCT emulsions were eliminated more rapidly than LCT emulsions, the former resulted in a greater increase in plasma concentrations of triacylglycerols and free glycerol than did the letter. This is explained by the fact that MCT provides about 1.8 times more triacylglycerol molecules than the LCT. In vitro, LPL and HL hydrolyzed structured lipids (randomly esterified triacylglycerols of medium- and long-chain fatty acids) slightly less rapidly then they did control lipids, but there was no comparable difference in the blood lipid parameters examined in vivo. Because the MCT emulsions are cleared rapidly, their fatty acids are rapidly made available to the various tissues where they are oxidized.

IN MAMMALS, circulating triacylglycerols cannot cross biologic membranes. For these neutral lipids to move from their carrier (chylomicrons, very-low-density lipoproteins [VLDL], or fat droplets that have been infused in the form of a phospholipid-stabilized emulsion) into the tissues, they have to be hydrolyzed with the release of fatty acids. This hydrolysis is performed by two enzymes: lipoprotein lipase (LPL; EC 3.1.1.34),[1] which is located at the capillary endothelium in extrahepatic tissues, and hepatic lipase (HL; EC 3.1.1.3).[2] The hydrolytic activity of these two enzymes provides the major pathway by which endogenous or exogenous triacylglycerol-rich particles are cleared from the bloodstream.

Parenteral nutrition frequently includes a supply of lipids in the form of an emulsion. At present, two types of emulsion are commercially available, distinguished by the nature of the oil used. It is generally a vegetable oil (soybean or safflower oil; LCT) the constituent triacylglycerols of which have long-chain fatty acids (longer than C_{12}, LCFA). Recently, emulsions have become available that provide medium-chain triglycerides (MCT) (constituent fatty acids between C_6 and C_{12}, MCFA). The specific physicochemical properties of MCT and MCFA[3] have led more and more clinicians to introduce this type of emulsion into the parenteral nutrition of their patients.[4] Our aim here was to investigate in rats how the nature of the oil used to make the emulsion affects the rate of clearance of this emulsion in vivo and the activity of the two enzymes responsible for most of the clearance.

MATERIALS AND METHODS

Intravenous Fat Tolerance Test

Between 1 and 3 PM male rats (OFA strain, from Iffa-Credo, l'Arbresle, France), weighing approximately 400 g, were anesthetized with sodium pentobarbital (40 to 50 mg/kg body weight; Clin Midy, Saint Jean de la Ruelle, France). A catheter (Intramedic PE 50, RUA Instruments, Torcy, France) was introduced into the jugular vein and another (Silastic medical grade tubing, Dow Corning, 602 135, Sigma Medical, Nanterre, France) into a femoral artery. The first catheter was maintained with a 9 g/L NaCl infusion (4.5 mL/h for 15 minutes before and 1.2 mL/h after the fat infusion) and the second filled with a citrate buffer, pH 7.4. The animals were not given heparin. The lipid emulsion was infused through the jugular vein (12 mL/h) (B. Braun Perfusor, Melsungen, West Germany) for four minutes. The amount of lipids represented 0.8 mL of emulsion. For rats weighing approximately 400 g, this represents approximately 0.4 g triacylglycerols/kg body weight. Arterial blood was sampled before the infusion of the emulsion, then at five-minute intervals for 40 to 60 minutes. The samples (700 to 750 μL at 0 and 40 minutes; otherwise 100 to 150 μL) were collected by simple flow onto dried anticoagulant (Anticlot, Fumouze, Ile Saint-Denis, France). The hematocrit went from 48% ± 0.5% at 0 minutes to 47% ± 0.6% at 40 minutes ($n = 30$, NS).

Immediately after the blood sample was collected, part of it was suitably diluted[5,6] in mannitol (50.7 g/L) mixed carefully, and centrifuged (620 × g for 15 minutes) (2KD centrifuge, Sigma, Osterode am Harz, West Germany). The supernatant was transferred to a cuvette, and its light-scattering index (LSI)[7] was determined (arbitrary units) at 650 nm as soon as possible by means of a nephelometer (Photovolt Corporation, NY).

This method of measuring the light scattered by the suspended fat particles made it possible to evaluate the amount of exogenous lipids present in the circulating blood. From the measurements taken each five minutes, we deduce the value at 0 minutes. The net values follow a straight line when plotted on a semilogarithmic scale.

The rest of the blood was also diluted and then centrifuged (at 620 × g for 15 minutes), and the plasma was decanted and frozen at −20°C until the moment of estimation of free glycerol[8] and triacylglycerols (Triglycerides enzymatique, Trinder, Biotrol, Paris, France; free glycerol is substrated from total glycerol). Nonesterified fatty acid (NEFA) levels were measured by an enzymatic method (Wako Nefa C test, Biolyon, Dardilly, France), which is applicable to MCFA. The expression of the calibration curve of $C_{8:0}$ is $y = 0.95x - 1.92$. Hematocrit determinations permitted the

From Laboratoire de la Clinique Médicale A, Strasbourg, France

Address reprint requests to Dr Olivier Lutz, Laboratoire, Clinique Médicale A, Hôpital Civil 67091, Strasbourg Cédex, France.

0026-0495/89/3806-0003$03.00/0

expression of the results obtained in terms of concentration per liter of plasma. At times 0 and 40 minutes, 600 μL of whole blood was precipitated with $HClO_4$ for estimation of the β-hydroxybutyrate.[9]

Measurement of the Activity of Diaphragm Lipoprotein Lipase

The activity of the LPL from rat diaphragms was measured using an adaptation of Boulangé's method.[10] The diaphragm was removed from freshly decapitated male rats, quickly rinsed in NH_4Cl, dried, and then ground (for 15 seconds in an Ultra-Turrax grinder, Janke and Kunkel, Staufen, West Germany) in cold NH_4Cl/heparin (50 mmol/L, pH 8.6, containing 4 IU heparin/mL; 1 g tissue per 12 mL NH_4Cl/heparin). After 15 minutes of contact at 4°C, the homogenate was centrifuged (15 minutes at 2,600 × g in the 2KD centrifuge). The supernatant was used unmodified in the case of the LCT emulsions and was diluted to ½ or ⅓ with NH_4Cl/heparin in the case of MCT emulsions.

To measure the LPL activity, 0.3 vol of a 20% emulsion was preincubated for 30 minutes at 4°C with 1.2 vol of inactivated rat serum (30 minutes at 56°C). Then 2.6 vol of albumin (190.5 g/L, pH 8.6; fraction V, Sigma, St Louis) and 0.9 vol of glycine (100 mmol/L, pH 8.6) were added to the incubation medium. After addition of 5.0 vol of diaphragm extract, the incubation medium was stirred and heated to 37°C.

At various times, the reaction was stopped with ice-cold benzeneboronic acid (final concentration 0.8 mmol/L) (EgA-Chemie, Steinheim, Aldrich, West Germany).[11,12] The mixture was centrifuged twice (two minutes at 9,000 × g; 3200 centrifuge, Eppendorf, Hamburg, West Germany) to eliminate lipid particules by flotation. On the underlying liquid we estimated in duplicate the free fatty acids. Benzeneboronic acid had no effect on the estimation of fatty acids per se.

The release of free fatty acids was linear with time for at least 60 minutes. A control incubation, in which the tissue extract was replaced with NH_4Cl-heparin was run in parallel with each experiment. The measured activity, expressed as μmol free fatty acids released/g muscle/h, was taken to be LPL activity because it was inhibited by either the omission of serum Apo C_{II}, the addition of NaCl, or of protamine sulfate (Serva, Heidelberg, West Germany) as shown by others.[13-15]

Measurement of Hepatic Lipase Activity

Male rats were anesthetized and their livers were perfused (Watson-Marlow perfuser, Falmouth, Cornwall, England) in situ according to the method of Hems et al.[16] The liver was rinsed initially with 200 mL Krebs-Henseleit bicarbonate buffer, pH 7.4, over a period of about ten minutes, without recycling. It was then perfused for six minutes with Krebs-Henseleit/heparin (1 mL/10 IU) at 60 mL buffer/kg body weight. This time the perfusate was recycled, and its was then centrifuged and divided into aliquots that were kept at −80°C until use. To 0.3 vol of a 20% lipid emulsion, we added 2.6 vol of bovine albumin (190.5 g/L, pH 8.6), 6.3 to 6.7 vol of 100 mmol/L glycine (pH 8.6), and 0.4 to 0.8 vol enzyme suspension. The procedure for testing HL activity was the same as that for LPL. The release of free fatty acids was linear with time for 60 minutes. In a parallel control test, the enzyme extract was heated in advance to 100°C for two minutes. The HL activity was expressed as micromoles of free fatty acids released/g liver/h. NaCl and protamine sulfate had little effect on HL activity.[15,17,18]

Emulsions

Table 1 shows the compositions of the various emulsions used. Structured lipids (SL-MCT/LCT) are randomly esterified triacylglycerols of MCFA and LCFA.

RESULTS

Intravenous Fat Tolerance Test

In our experimental conditions we obtained a biphasic elimination curve (Fig 1), indicating zero-order kinetics for removal at high fat concentrations and first-order kinetics at lower substrate concentrations. With the change from LCT to MCT, the clearance rate increased and the rise of β-hydroxybutyrate (between 0 and 40 minutes the increase was, respectively, + 209 ± 21 μmol/L for LCT ν + 702 ± 50 for MCT) was greater. The increase in circulating lipids was also greater (Fig 1). If with LCT, and to a lesser extent with MCT, the curve of LSI variation appeared as being more abrupt than those of plasma lipids, it is explained both by the difference in the expression of results (as concentration in the case of LSI; as percentage of infused fat in the case of plasma lipids) and by the intervention in the clearance of a supplementary mechanism, the uptake by the reticuloendothelial system.[4]

A comparison of the results obtained after the infusion of a 20% 70/30 (wt/wt) MCT/LCT emulsion with those obtained after infusion of a 70/30 structured lipid emulsion (Fig 2) shows that the clearance rates are identical as are the changes in plasma lipids and blood β-hydroxybutyrate (+425 ± 40 μmol/L for MCT/LCT ν +408 ± 29 for SL-MCT/LCT) levels.

In Vitro Experiments

An MCT emulsion was more rapidly hydrolyzed by LPL or HL than was an LCT emulsion (Fig 3). In the case of a mixed MCT/LCT emulsion, the activity of the two enzymes went up as the percentage of MCT increased (Fig 4). For LPL the V_{max} and K_m were, repectively, 70 μmol/g/h and 3.67 mmol/L for the 20% LCT emulsion, and 154 μmol/g/h

Table 1. Composition of the Emulsions Used

Lipids	Trade Name or Batch Number	Manufacturer	Oils (g/L)
LCT	Intralipid 20% or 10%	Kabi-Vitrum	Soybean oil (200 or 100)
	Endolipide 20% or 10%	Bruneau	Soybean oil (200 or 100)
MCT/LCT	Lipofundin MCT, 50/50 20% or 10%	B. Braun Melsungen	Soybean oil (100 or 50) + MCT (100 or 50)
	MCT/LCT, 70/30, 20% (V. 12 274)	B. Braun Melsungen	Soybean oil (60) + MCT (140)
	SL-MCT/LCT 70/30, 20% (V. 12 275)	B. Braun Melsungen	Soybean oil (60) + MCT (140); Structured lipids
MCT	MCT 20% (CH E 86024)	B. Braun Melsungen	MCT (200)
	MCT 20% (OLG000 R2-PXE0815)	Travenol	MCT (200)

Egg phosphatides (12 g/L) were always added as an emulsifier and glycerol (22.5 g/L for Intralipid; otherwise 25 g/L) as an isotonic adjuvant.

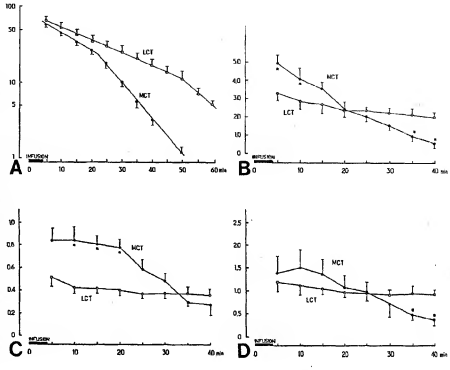

Fig 1. Effect of the nature of the oil in the infused emulsion on the circulating lipids. (A) LSI (arbitrary units), (B) triacylglycerols (% of infused fat/mL plasma), (C) free glycerol (% of infused fat/mL plasma), (D) NEFA (% of infused fat/mL plasma). Results are expressed as mean ± SEM (n = 8 for LCT, 6 for MCT). Bars represent 1 SEM. Triacylglycerol levels were corrected for free glycerol. The rats were deprived of food at 9 AM and tested between 1 and 3 PM. LCT, Intralipid; MCT, OL6000 R2. Except for the LSI figure, serum levels at a given time less the initial level are given (expressed as μmol triglycerides, NEFA, or free glycerol) as a percentage of the amount of infused fats.* The difference between the two means at a given time is highly significant (Student's *t*-test, $P < .05$).

and 3.55 mmol/L for the 20% MCT emulsion. For HL these values were, respectively, 15.5 μmol/g/h and 3.27 mmol/L for the 20% LCT emulsion and 18.2 μmol/g/h and 0.61 mmol/L for the 20% MCT emulsion. The activity of LPL, similar to that of HL, increased with increasing amounts of albumin in the incubation medium; maximum activity was reached more quickly with MCT than with LCT (Fig 5A,B). The same was true with the inactivated serum in the case of LPL (Fig 5C,D). The activity of HL toward LCT decreased greatly in the presence of serum, whereas toward MCT it increased slightly. The hydrolysis of structured lipids was slightly slower than that of the corresponding MCT/LCT

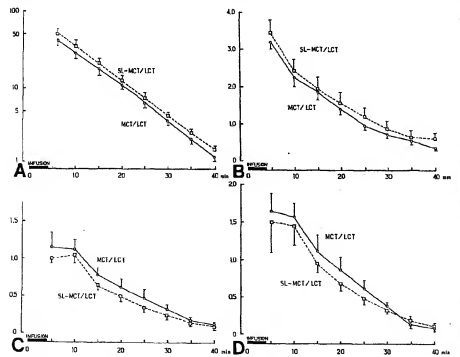

Fig 2. Effect of a physical v e chemical (structured) MCT/LCT mixture on the circulating lipids. (A) LSI (arbitrary units), (B) triacylglycerols (% of infused fat/mL plasma), (C) free glycerol (% of infused fat/mL plasma), (D) NEFA, (% of infused fat/mL plasma). Results are expressed as mean ± SEM (n = 9 for MCT/LCT, and 8 for SL-MCT/LCT). Bars represent 1 SEM. Triacylglycerol levels were corrected for unesterified glycerol. The rats were not fasted. MCT/LCT, V.12 274; SL-MCT/LCT, V.12 275. Except for the LSI figure, serum levels at a given time less the initial level are given (expressed as μmol triglycerides, NEFA, or free glycerol) as a percentage of the amount of infused fats.

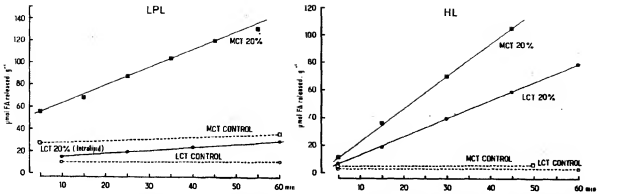

Fig 3. Hydrolysis of fat emulsion by LPL and HL as a function of time. FA, fatty acids. LCT 20 %, Endolipide; MCT, OLG000 R2. Incubation mixtures: LPL: 30 μL 20% emulsion; 260 μL albumin; 120 μL serum; 90 μL glycine; 500 μL enzyme in NH_4Cl/heparin. HL: 30 μL 20% emulsion; 260 μL albumin; 660 μL glycine; 50 μL enzyme in Krebs-Henseleit buffer.

mixture (Fig 6). With LPL the K_m was 8.51 mmol/L with the structured MCT/LCT (SL-MCT/LCT) preparation and 4.78 mmol/L with MCT/LCT, whereas the V_{max} values were, respectively, 98 and 117 μmol/g/h. For HL the respective values were K_m, 1.23 and 1.57 mmol/L and V_{max}, 12.6 and 15.4 μmol/g/h.

DISCUSSION

Long- and Medium-Chain Triglycerides

It is well known that LPL hydrolyzes triacylglycerol-rich lipoproteins[19] and particles of artificial fat emulsions.[20,21] Likewise, HL undeniably shows a triacylglycerol lipase activity in vitro.[18,22] The results shown in Fig 3 point in the same direction: the LCT- or MCT-based fat emulsions are hydrolyzed in vitro, both by LPL[22] and by HL. These results also widen the applicability of the observation made by Wang et al[24] on human milk LPL: muscle LPL hydrolyzed MCT emulsions more rapidly than it did LCT emulsions. The same was true of HL (Fig 3). Mixed MCT/LCT emulsions lay between LCT and MCT (Fig 4). The release of fatty acids by LPL and HL became faster as the percentage of MCT in the mixture increased.

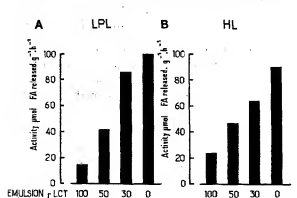

Fig 4. Effects of the relative quantities of MCT and LCT in the 20% emulsion on the activity of LPL (A) and of HL (B). FA, fatty acids; LCT, Endolipide; MCT/LCT 50/50, Lipofundin MCT; MCT/LCT 70/30, V.12 274; MCT ch E 86 024. Incubation mixtures: LPL: 30 μL 20% emulsion; 120 μL serum; 260 μL albumin, 90 μL glycine; 500 μL enzyme in NH_4Cl/heparin. HL: 30 μL 20% emulsion; 260 μL albumin; 70 μL enzyme in Krebs-Henseleit buffer; 640 μL glycine.

The faster hydrolysis of MCT by LPL is confirmed by the V_{max} value, which was 2.2 times larger for MCT than for LCT. The ratio V_{max} MCT/V_{max} LCT was only 1.2 in the case of HL. With regard to the LPL affinity ($1/K_m$), it is the same for both substrates (ratio MCT/LCT = 1.0); HL affinity, on the contrary, was widely in favor of MCT (ratio 5.4). Several hypotheses can be proposed to explain these results: (1) The ester bond is weaker in MCT.[1] (2) The interfacial tension of oil against water being less in the case of MCT than for the LCT, the MCT based emulsions are more fragile. The faster destruction of the MCT in the presence of NaCl is consistent with this fact. (3) A difference in the surface area between the two types of fat particles allows the enzyme to better hydrolyze the MCT-based emulsions. (4) The existence of a retroinhibition of enzymes by the released fatty acids would be stronger with LCFA.

Structured Lipids

High hopes are held for structured lipids,[25] emulsions of which are thought to bring about a positive nitrogen balance in parenterally fed rats more rapidly than the currently used commercial emulsions.[26] In a 70/30 (wt/wt) MCT/LCT emulsion each lipid particle contains molecules of both MCT and LCT. Because their mean molecular weights are, respectively, 492 and 872, 81% of the triacylglycerol molecules possess an MCFA in position sn-1 (as well as in positions 3 and 2). This is not the case with structured lipids. These triacylglycerols contain in the majority both MCFA and LCFA in the same molecule. Considering the initial proportions (70/30 wt/wt) and the random distribution of fatty acids, it is safe to say that the majority of triacylglycerol molecules of the SL-MCT/LCT emulsion contain two MCFA for one LCFA. Because in certain of these triacylglycerols, the LCFA occupies the position sn-1, one can assume that approximately 66% (v 81% in MCT/LCT) of the MCFA are in a favored position for action by the LPL.[1] We observed that these emulsions were hydrolyzed by the two lipases involved in the catabolism of triacylglycerol-

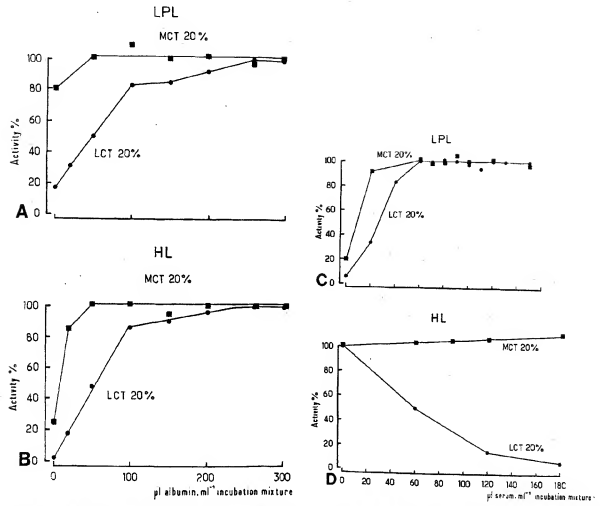

Fig 5. Dependence of LPL and HL activity on the albumin (A, B) or inactivated serum (C, D) concentrations in the mixture. LCT, intralipid; MCT, ch E 86 024. The observed activity with 260 µL of albumin (A, B) and 120 µl (for LPL) — 0 µL (for HL) serum (C, D) was arbitrarily taken as 100%. Incubation mixtures: LPL: 30 µL 20% emulsion; 120 (A) or 0–180 (C) µL serum; 0–300 (A) or 260 (C) µL albumin; 500 µL enzyme in NH_4Cl/heparin; the medium was made up to 1 mL with glycine solution. HL: 30 µl 20% emulsion; 0 (B) or 0–180 (D) µL serum; 0–300 (B) or 260 (D) µL albumin; 100 (B) or 40 (D) µL enzyme in Krebs-Henseleit buffer; the medium was made up to 1 mL with glycine solution.

rich particles, but slightly more slowly than the usual MCT/LCT emulsions (Fig 6). The V_{max} ratio for MCT/LCT *v* SL-MCT/LCT was 1.2, ie, identical to that of 81/66, the ratio of the MCFA molecular quantities in the favored position in the two emulsions. LPL had a higher affinity for the physical mixture (ratio MCT/LCT *v* SL-MCT/LCT = 1.8), whereas HL seemed to prefer the structured lipids (ratio 0.8).

Albumin Requirement

Because fatty acids released by the enzymatic action on an LCT emulsion are not water-soluble, serum albumin must be added to bind these components and to allow hydrolysis to proceed. This is indeed the case with LCT, both for LPL[3,27,28] and HL[72] (Fig 5A,B). It is also true of MCT, but in this case less albumin is needed, and the maximum activity is reached with lower concentrations of albumin. This observation may be explained by the difference in solubility of the various fatty acids in water: at 20°C the solubility is 7.2 mg/L (28 µmol/L) for palmitic acid *v* 680 mg/L (4,716 µmol/L) for octanoic acid.[29] Tributyrin is hydrolyzed by milk LPL in the absence of albumin.[30]

Serum Requirement

For maximum activity, LPL requires the presence of apolipoprotein C_{II}.[3-15,23,31,32] If an artificial emulsion is used, it must first be activated by incubation with serum. Figure 5C,D shows that the rate of triacylglycerol hydrolysis by LPL increased as a function of serum concentration and that the maximum activity of LPL on MCT is reached with less serum and hence less apoC_{II} than is needed for LCT. It had been shown previously that the enzymatic hydrolysis of

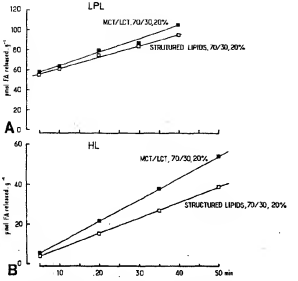

Fig 6. Hydrolysis of MCT/LCT structured lipids by LPL (A) and HL (B) FA, fatty acids; MCT/LCT, V. 12 274; structured lipids, V.12 276. Incubation mixtures are as indicated in Fig 3.

tributyrin, is not $apoC_{II}$-dependent.[23,28,30] Although $apoC_{II}$ is a specific activator for LPL, it is an inhibitor of HL.[33-35] We also observed that serum strongly inhibits HL, but only in the case of the LCT emulsion (Fig 5C,D), and not at all with the MCT emulsion. It is thus probable that in vivo the liver plays an important role in clearing MCT-based fat emulsions. The hyperketonemia observed after MCT infusion can be explained partially by this fact.

Fate of Infused Fats

The difference in activity between LPL and HL toward MCT and LCT emulsions explains what is seen in vivo (Fig 1): the former are cleared more rapidly than the latter, as has previously been shown in metabolically healthy adults[36,37] and in dogs.[38] Although MCT were cleared more rapidly than LCT, our results show that the plasma concentration of triacylglycerols increased more with MCT than with LCT.[36,39,40] This happens because for a given weight of emulsion, the two types of lipids do not provide the same number of fat molecules. Considering the mean molecular weight of the triacylglycerols used, the MCT provide approximately 1.8 times more triacylglycerol molecules than the LCT. The same explanation applies to glycerol, the plasma concentration of which (Fig 1) increased the most following infusion with MCT. The increase in NEFA following the infusion of MCT did not differ significantly from that following LCT, but the difference was significant during the disappearance phase of the curve; at 35 and 40 minutes the NEFA were lower for MCT than for LCT. One explanation is that the half-life of MCFA is shorter than the half-life of LCFA.

During an MCT infusion, we theorize a larger role of liver in exogenous fat clearance, and observe a higher increase of circulating fatty acids. Since the work of Scheig,[41] it has been known that in the liver MCFA, unlike LCFA, are poorly incorporated into lipids and are strongly oxidized. The resulting increase in acetyl-CoA is greater with MCT than with LCT.[42] This substantial β-oxidation of MCFA may explain the great increase in blood ketone levels after infusion of an MCT-based emulsion.[36,37,39,40] Our results show that the amount of circulating β-hydroxybutyrate rose more with MCT than with LCT. In vivo the 70/30 MCT/LCT and 70/30 SL-MCT/LCT emulsions behaved indistinguishably. Clearance and changes in plasma lipid and blood β-hydroxybutyrate concentrations[26] were the same (Fig 2), whereas in vitro the activity of the two enzymes involved in their clearance was a little greater with the "physical" mixture than with the "chemical" mixture (Fig 6). Because LPL and HL hydrolyze an MCT-based emulsion rapidly, these triacylglycerols are cleared more rapidly than an LCT emulsion. MCFA, which, not being stored, are energy substrates par excellence, are therefore more rapidly made available to the tissues of a living body than are the LCFA of a traditional emulsion.

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